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REVIEW ARTICLE

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Aspirin-Intolerant Asthma (AIA) Assessment Using the Urinary Biomarkers, Leukotriene E_4 (LTE_4) and Prostaglandin D_2 (PGD_2) Metabolites

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ABSTRACT
The clinical syndrome of aspirin-intolerant asthma (AIA) is characterized by aspirin/nonsteroidal anti-inflammatory drug intolerance, bronchial asthma, and chronic rhinosinusitis with nasal polyposis. AIA reactions are evidently triggered by pharmacological effect of cyclooxygenase-1 inhibitors. Urine sampling is a non-invasive research tool for time-course measurements in clinical investigations. The urinary stable metabolite concentration of arachidonic acid products provides a time-integrated estimate of the production of the parent compounds *in vivo*. AIA patients exhibits significantly higher urinary concentrations of leukotriene E_4 (LTE_4) and 1,15-dioxo-9α-hydroxy-2,3,4,5-tetranorprostan-1,20-dioic acid (tetranor-PGDM), a newly identified metabolite of PGD_2, at baseline. This finding suggests the possibility that increased mast cell activation is involved in the pathophysiology of AIA even in a clinically stable condition. In addition, lower urinary concentrations of primary prostaglandin E_2 and 15-epimer of lipoxin A_4 at baseline in the AIA patients suggest that the impaired anti-inflammatory elements may also contribute to the severe clinical outcome of AIA. During the AIA reaction, the urinary concentrations of LTE_4 and PGD_2 metabolites, including tetranor-PGDM significantly and correlatively increase. It is considered that mast cell activation probably is a pathophysiologic hallmark of AIA. However, despite the fact that cyclooxygenease-1 is the dominant *in vivo* PGD_2 biosynthetic pathway, the precise mechanism underlying the PGD_2 overproduction resulting from the pharmacological effect of cyclooxygenease-1 inhibitors in AIA remains unknown. A comprehensive analysis of the urinary concentration of inflammatory mediators may afford a new research target in elucidating the pathophysiology of AIA.

KEY WORDS
aspirin, asthma, biomarker, cysteinyl-leukotriene, mast cell, prostaglandin D_2, urine

ABBREVIATIONS
AIA, Aspirin-intolerant asthma; NSAID, Nonsteroidal anti-inflammatory drug; CRSwNP, Chronic rhinosinusitis with nasal polyposis; COX, Cyclooxygenase; CysLT, Cysteinyl-leukotriene; PGD_2, Prostaglandin D_2; LTE_4, Leukotriene E_4; HPLC, High-performance liquid chromatography; ATA, Aspirin-tolerant asthma; 15-epi-LXA_4, 15-Epimer of lipoxin A_4; IgE, Immunoglobulin E; LXA_4, Lipoxin A_4; 9α,11β-PGF_2, 9α,11β-Prostaglandin F_2; 2,3-Dinor-9α,11β-PGF_2, 2,3-Dinor-9α,11β-prostaglandin F_2; Tetranor-PGDM, 11,15-Dioxo-9α-hydroxy-2,3,4,5-tetranorprostan-1,20-dioic acid; EIA, Enzyme immunoassay; *ent*-$PGF_{2α}$, Prostaglandin $F_{2α}$ enantiomer;

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GPCR, G-protein coupled receptors; PPAR-γ, Peroxisome proliferator activated receptor γ; BMMC, Bone marrow-derived mast cell; 15R-PGD2, 15R-methyl-prostaglandin D2; DP2, Prostaglandin D2 receptor 2; GSH, Glutathione.

INTRODUCTION

The clinical syndrome of aspirin-intolerant athma (AIA) is characterized by aspirin/nonsteroidal anti-inflammatory drug (NSAID) intolerance, bronchial asthma and chronic rhinosinusitis with nasal polyposis (CRSwNP).[1,2] Aspirin/NSAID-induced asthma reactions are triggered by pharmacological effect of cyclooxygenease-1 (COX-1) inhibitors, whereas COX-2-specific inhibitors (coxibs) are tolerated in the vast majority of cases.[3,4] Several clinicoepidemiologic studies[5-9] have demonstrated that AIA is one of the common risk factors for the development of refractory asthma. Previous *in vitro* studies demonstrated the release of chemical mediators from leukocytes in AIA patients and these *in vitro* tests may be applicable to a diagnosis of aspirin/NSAIDs intolerance.[10-13] However, there is no experimental evidence which directly supports this hypothesis.[14,15] That is, there is neither an appropriate *in vitro* test to diagnose AIA nor an animal model to help fully elucidate the pathogenesis of AIA. Thus, biological fluid samples from AIA subjects are the only research tools available. Urine samples constitute a non-invasive research tool for time-course measurements in the clinical setting, although such data does not provide any information on the sites of the production. The urinary concentration of chemical mediator metabolites is remarkably

Table 1 Inflammatory mediators in biological samples

Invasive --► Non-invasive

<table>
<tr><th>BALF</th><th>ELF</th><th>Induced sputum</th><th>Plasma</th><th>EBC</th><th>Saliva</th><th colspan="2">Urine</th></tr>
<tr><td colspan="3">unmetabolized compounds</td><td>metabolites</td><td colspan="3">unmetabolized compounds</td><td>urinary metabolites</td></tr>
<tr><td colspan="3">local production</td><td>whole body production</td><td colspan="3">Local production</td><td>whole body production</td></tr>
<tr><td colspan="7">instantaneous measure of endogenous production of the compounds</td><td>time-integrated measure of endogenous production of the compounds</td></tr>
<tr><td></td><td>ext. high levels</td><td></td><td>ext. Low levels</td><td>Low levels</td><td></td><td colspan="2">relatively high levels</td></tr>
</table>

Abbreviations: BALF, bronchoalveolar lavage fluid; ELF, epithelial lining fluid; EBC, exhaled breath condensate; ext., extremely.

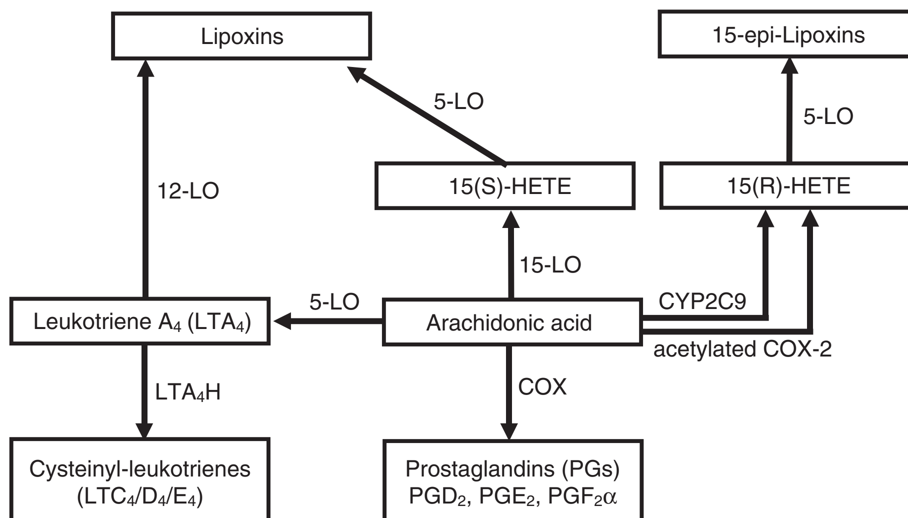

Fig. 1 Arachidonic acid cascade (Adapted from Yamaguchi *et al*.[49]).

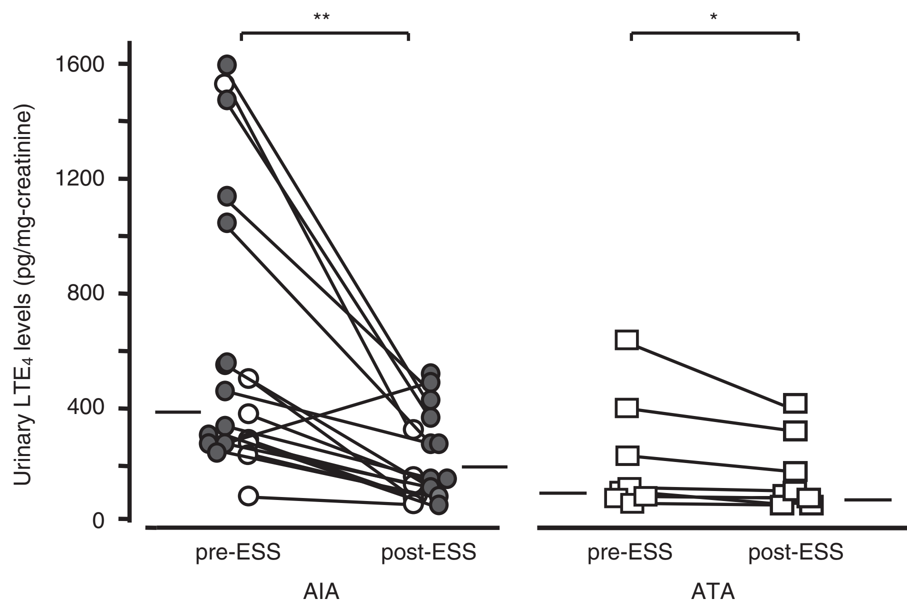

Fig. 2 Changes in the urinary LTE_4 concentrations following endoscopic sinus surgery (ESS). *Horizontal bars* indicate medians. Patients with aspirin-intolerant asthma (AIA, n = 19) and patients with aspirin tolerant asthma (ATA, n = 8) are denoted by *circles* and *squares*, respectively. Additional urinary data (12 AIA patients, closed *circles*) were included in the original figure published previously.[26] The asterisks (*, **) indicate $p < 0.05$ and $p < 0.01$, respectively, in comparison with baseline values (pre-ESS).

higher than the plasma concentration, which allows us to correlate the mediator metabolite levels with clinical symptoms. The characteristics of the biological fluid samples are shown in Table 1. It is important to note that because various arachidonic acid metabolites (Fig. 1) are rapidly metabolized *in vivo*, the urinary concentration of their stable metabolites provides a time-integrated estimate of the production of the parent compounds, allowing a detection of their generation *in vivo*.[16] In this review article, we focus on the clinical implications of urinary biomarkers such as cysteinyl-leukotrienes (CysLTs) and prostaglandin D_2 (PGD_2) metabolites in AIA.

URINARY LEUKOTRIENE (LT) E_4 IN AIA AT BASELINE

Since the leukotriene (LT) C_4 is easily metabolized in the lungs and/or the liver, and then disappears into the body fluid, LTE_4 is the predominant metabolite among the CysLTs of defined structure.[17,18] It is reported that 4-13% of the intravenous or inhaled dose of LTC_4 is excreted in the urine.[16,19,20] Arachidonic acid metabolites are present in extremely small quantities in biological fluids (on an order ranging from pg/ml to ng/ml). When assayed by enzyme immunoassay (EIA), biological samples should be purified in order to eliminate any interfering substances. Thin-layer chromatography has been commonly employed to remove such interfering substances. We believe that purification using high-performance liquid chromatography (HPLC) is also convenient and provides a suitable quantification procedure. Recently it has been emphasized that additional chromatographic steps are required for obtaining reliable data on the urinary LTE_4 concentrations.[21,22] There is increasing evidence that the AIA group exhibits a significantly higher urinary LTE_4 excretion level at baseline than the aspirin-tolerant asthma (ATA) group, even in a clinically stable condition.[23-31] Of late it appears that the baseline urinary LTE_4 concentrations in the AIA group are on a decline compared with original data by Christie *et al*.[23] and Kumlin *et al*.,[29] perhaps because of a stabilization of asthma symptoms by inhaled corticosteroids. Considering the distinct evidence from several immunohistochemical studies,[32-34] LTC_4 producing cells such as eosinophils and mast cells seem to contribute to increased baseline concentrations of urinary LTE_4 in subjects with AIA. Interestingly, we have demonstrated that a severe ATA group with chronic rhinosinusitis with nasal polyposis (CRSwNP) also exhibited a significantly higher urinary LTE_4 concentration at baseline.[26] That is, CysLT overproduction is associated with the clinical features of severe asthma with CRSwNP, that is, the so-called “aspirin triad” in AIA. Taken together with the evidence that there is a close relationship between CRSwNP and CysLT overproduction in asthmatic subjects, we have proposed the concept of “hyper-leukotrienuria”.[26,35] The preliminary data indicates that aspirin intolerance seldom develops in pa-

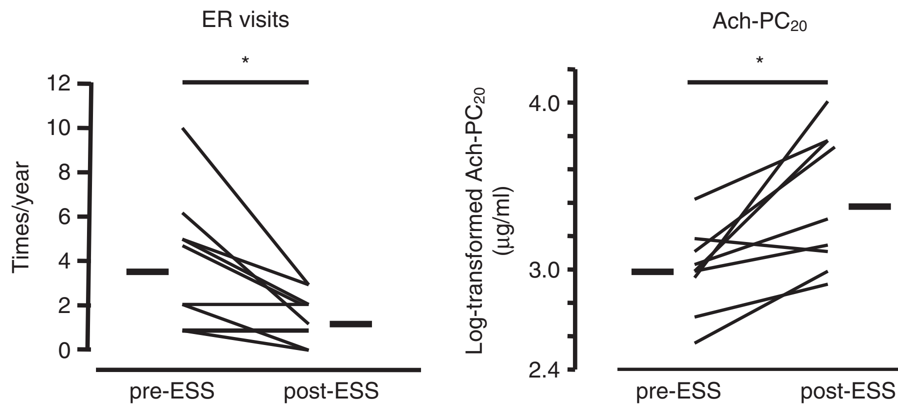

Fig. 3 Evidence of clinical improvement of asthma symptoms after endoscopic sinus surgery (ESS). *Horizontal bars* indicate means. The asterisk (*) indicates $p < 0.05$ in comparison with baseline values (pre-ESS, $n = 9$).

Table 2 Analytical significance of urinary eicosanoid concentrations

Urinary eicosanoids	Metabolites compounds				Unmetabolites compounds	
	LTE_4	D-ring PGDM	F-ring PGDM	PGEM and tetranor-PGEM	primary PGE_2	*ent*-$PGF_{2}\alpha$
Derived from	5-LO	COX (COX-1 dominant)		COX (COX-2 dominant)		non-enzymatic
As an index of	whole body production				local kidney production	free radical-mediated production
	LTC_4	PGD_2		PGE_2		

Abbreviations: LTE_4, leukotriene E_4; 5-LO, 5-lipoxygenase; PGDM, prostaglandin D_2 metabolite; F-ring PGDM, 9α,11β-prostaglandin F_2 and 2,3-Dinor-9α,11β-prostaglandin F_2; D-ring PGDM, tetranor-PGDM; PGE_2, prostaglandin E_2; PGEM, 13,14-dihydro-15keto-prostaglandin E_2; tetranor-PGEM, 9,15-dioxo-11α-hydroxy-2,3,4,5-tetranor-prostane-1,20-dioic acid; COX, cyclooxygenase; *ent*-$PGF_{2}\alpha$, prostaglandin $F_{2}\alpha$ *enantiomer*.

The original table has been published previously with some modification.[25]

tients with ATA, even with hyper-leukotrienuria, throughout the course of a 5-year follow-up period (unpublished data). This clinical finding leads us to the hypothesis that CysLT overproduction *in vivo* does not promote aspirin intolerance by itself. Interestingly, it was demonstrated for the first time that there is a significant decrease in the urinary LTE_4 concentrations after endoscopic sinus surgery in both the AIA and ATA groups. We carried out further investigation in 12 additional AIA patients, and found further evidence which suggests that CRSwNP is involved in CysLT overproduction in asthmatic subjects, as shown in Figure 2. Furthermore, we have preliminarily determined the clinical improvement in asthma-related emergency room visits and bronchial hyperresponsiveness after endoscopic sinus surgery. Unexpectedly, sinus surgery resulted in significantly fewer asthma-related emergency room visits. In addition, there were significant increases in the Ach-PC_{20} values after endoscopic sinus surgery. (unpublished data, Fig. 3) These data typically support the concept of "one airway, one disease".[36] Recent research suggests a close relationship between LT biosynthesis and vascular events such as arteriosclerosis.[37,38] We have reported significantly increased urinary LTE_4 concentrations in patients with acute exacerbated vasculitides.[39] It is also reported that the urinary LTE_4 concentration is slightly increased in current smokers[40] and obese subjects,[41] suggesting that the urinary LTE_4 concentration may be useful as a non-invasive biomarker of oxidative tissue inflammation and related pathophysiologic events.[42]

URINARY PGs CONCENTRATIONS IN AIA AT BASELINE

Recently we reported that the urinary PGE_2 concentrations at baseline in the AIA group are significantly lower than the ATA group.[25] Since PGE_2 in urine is an unmetabolized compound, the primary PGE_2 concentrations in urine have been considered to predominantly reflect local renal production[43] (Table 2). Interestingly, lower spontaneous production of PGE_2 has been reported in epithelial cells from nasal polyps[44] and sinonasal tissue[45] in subjects with AIA,

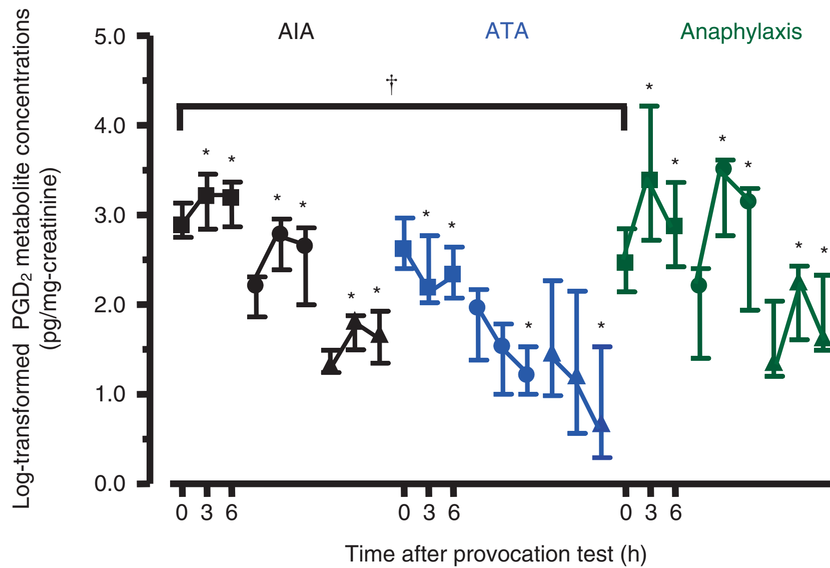

Fig. 4 Urinary PGD_2 metabolites following AIA and anaphylaxis reactions. The log-transformed urinary concentrations of PGD_2 metabolites are expressed as medians and interquartile ranges in the AIA (n = 10 or 8§), ATA (n = 7) and anaphylaxis (n = 8) groups, respectively. § In the case of tetranor-PGDM concentrations. The urinary tetranor-PGDM, 2,3-dinor-9α,11β-prostaglandin F_2 and 9α,11β-prostaglandin F_2 are denoted by *squares*, *circles* and *triangles*, respectively. The asterisk (*) indicates $p < 0.05$ compared with baseline values. A dagger (†) indicates $p < 0.05$ for the comparison between the AIA and anaphylaxis groups. Urine samples were collected at baseline and following the reactions.

which is associated with diminished COX-2 expression in these tissues.[46,47] Inhaled PGE_2 protects against both aspirin-induced bronchoconstriction and the massive release of urinary LTE_4,[48] so a critical deficiency in PGE_2 "braking" has been postulated as one possible mechanism for the AIA reaction. Similarly we have demonstrated a decreased urinary concentration of 15-epimer of lipoxin A_4 (15-epi-LXA_4),[49] which is also produced by cell-to-cell interaction involving acetylated COX-2 and 5-lipoxygenase.[50] In allergic airway inflammation, not only LXA_4 but also 15-epi-LXA_4 block both bronchial hyperresponsiveness and pulmonary inflammation induced by eosinophils via the LXA_4 receptor, leading to decreases in the numbers of eosinophils and T-lymphocytes and decreases in the concentrations of interleukin-5, interleukin-13, eotaxin, immunoglobulin E (IgE), PGs, and CysLTs.[51] Thus, it is a plausible explanation that the low COX-2 expression *in vivo* may result in the lower urinary concentrations of anti-inflammatory PGE_2 and 15-epi-LXA_4 at baseline in the AIA group, suggesting the deficiency of additional anti-inflammatory elements in AIA. Since the AIA subjects excreted significantly higher urinary LTE_4 concentration at baseline even in a clinically stable condition,[23-29] as described above, an imbalance between the local production of pro-inflammatory CysLTs and anti-inflammatory 15-epi-LXA_4 and PGE_2 at baseline, may play an important role in development of refractory asthma in AIA. Unexpectedly, urinary lipoxin A_4 (LXA_4) was significantly lower than 15-epi-LXA_4 because 15-epi-LXA_4 shows a two-fold longer half-life *in vivo*,[52] as calculated by conversion rate of 15-hydroxyprostaglandin dehydrogenase.

URINARY CONCENTRATIONS OF PG METABOLITES IN AIA AT BASELINE

The major urinary metabolites of PGE_2 and PGD_2 are shown in Table 2. Among LTC_4 producing cells such as eosinophils, basophils and mast cells, it is only the mast cells that produce significant quantities of PGD_2 in human.[53] Although there is evidence of some PGD_2 formation by eosinophils, platelets, macrophages and certain T lymphocytes, the reported amounts are 100 to 1000 times lower than those produced during IgE dependent activation of mast cells.[54-56] So the urinary PGD_2 metabolites are useful biomarkers of mast cell activation.[55] Although it is indeed also true that tryptase in the biological fluid samples is a mast cell-specific biomarker, it is impossible to detect tryptase in urine. Furthermore, it was reported that serum tryptase concentration was increased in only 6% of the patients who developed anaphylaxis.[57] Similarly, Bochenek *et al*. demonstrated that there was no change in serum tryptase despite the five-fold increase in plasma 9α,11β-PGF_2 follow-

ing the early phase of allergen-induced airway obstruction.[58] Thus, we concluded that the determination of the urinary PGD_2 metabolites is more sensitive and practical than serum tryptase for monitoring mast cell activation.[59] "F-ring" urinary PGD_2 metabolites, $9\alpha,11\beta$-prostaglandin F_2 ($9\alpha,11\beta$-PGF_2) and 2,3-dinor-$9\alpha,11\beta$-prostaglandin F_2 (2,3-dinor-$9\alpha,11\beta$-PGF_2), has been frequently used in the clinical studies.[25,55,60,61] Recently, Song *et al.* newly identified the most abundant "D-ring" PGD_2 metabolite in urine, 11,15-dioxo-9α-hydroxy-2,3,4,5-tetranorprostan-1,20-dioic acid (tetranor-PGDM).[61] We also examined the urinary tetranor-PGDM concentration with a newly commercially available enzyme immunoassay (EIA) kit (Cayman Chemical, Ann Arbor, MI, USA). Our data demonstrated that the urinary tetranor-PGDM concentrations were 4.8-fold (median) higher than 2,3-dinor-$9\alpha,11\beta$-PGF_2 or 11.7-fold higher than $9\alpha,11\beta$-PGF_2 at baseline.[62] Interestingly, the urinary concentrations of tetranor-PGDM and LTE_4 at baseline in the AIA group was significantly higher than the anaphylaxis group[62] (Fig. 4). To our knowledge, this is the first report of a significantly higher baseline concentration of PGD_2 metabolites in urine in AIA patients, except for patients with mastocytosis.[63] Thus, the quantification of tetranor-PGDM may be an attractive strategy for further investigation into mast cell activation. This finding is consistent with the previous reports of higher baseline concentration of sputum PGD_2,[27] plasma $9\alpha,11\beta$-PGF_2 and serum tryptase in the AIA patients.[64] Furthermore, it is reported that there was a significant increase in the number of submucosal mast cell in the bronchial biopsy obtained from AIA versus ATA patients.[65,66] It is important to note that the purification of urine by HPLC is crucial for the precise quantification of tetranor-PGDM. One limitation of this biomarker of tetranor-PGDM is that it is generally unstable when not stored at -80℃. By contrast, there is no significant difference in the major urinary PGE_2 metabolites, 13,14-dihydro-15keto-PGE_2 (PGEM) and 9,15-dioxo-11α-hydroxy-2,3,4,5-tetranor-prostane-1,20-dioic acid (tetranor-PGEM), at baseline between AIA and ATA groups,[67] in spite of the fact that COX-2 contributes substantially to the biosynthesis of PGE_2[68] and that lower COX-2 expression is one of the unique characteristics of AIA.[46,47] Interestingly, aspirin provocation decreases the urinary concentrations of PGE_2 metabolites in only the ATA group, not the AIA group.[67]

LTE_4 AND PG METABOLITES DURING ASPIRIN/NSAIDs-INTOLERANT ASTHMA REACTION

During systemic aspirin provocation, a runny nose and nasal congestion (>90%) are the first symptoms, and then acute bronchoconstriction develops more than 30 min after the administration of threshold dose of aspirin. This aspirin-induced bronchoconstriction almost never lasts beyond 24 hours. Extrapulmonary reactions such as skin reactions and gastrointestinal symptoms are minor complications.[69] The urinary LTE_4 concentrations after the systemic provocation test with aspirin in the AIA group significantly increased above the basal concentrations (approximately a 3-30-fold increase).[25,28,30,70,71] There is evidence that shows 1) excessive CysLTs overproduction *in vivo* during aspirin-induced bronchoconstriction is a pathophysiologic hallmark of AIA without exception, 2) an increase in urinary LTE_4 concentration after aspirin challenge, the most dramatic event in AIA, correlates with the severity of the aspirin-induced reaction and 3) there is no significant increase in the urinary LTE_4 concentration in ATA patients. Although CysLTs play a key role in the pathophysiology of aspirin/NSAID intolerance, LT receptor antagonists can attenuate but cannot completely block aspirin-induced asthmatic reactions.[72] One question that needs to be answered is which cells produce CysLTs during provocation test. Our study demonstrated the urinary concentrations of LTE_4 and PGD_2 metabolites (2,3-dinor-$9\alpha,11\beta$-PGF_2,[25] $9\alpha,11\beta$-PGF_2[25,28] and tetranor-PGDM[62]) were significantly increased and correlated with each other after the aspirin provocation test in the AIA group. Bochenek *et al.*[64] have demonstrated an increase in urinary $9\alpha,11\beta$-PGF_2 and serum tryptase after aspirin provocation in AIA patients. Increased concentrations of histamine and N^{τ}-methyhistamine, a urinary metabolite of histamine, have also observed after aspirin provocation in AIA patients.[28,73] Taken together, it is clear that mast cell activation is closely associated with aspirin-induced bronchoconstriction. However, markedly different patterns of CysLTs and PGD_2 release in the aspirin-intolerant asthma group and the IgE-mediated anaphylaxis group,[25] respectively, leads us to speculate that there may be other, unknown cellular sources besides mast cells for CysLTs synthesis during acute AIA reactions. Because eosinophils also have the capacity to generate both LTC_4 in large quantity and PGD_2 in much smaller quantity,[74-77] eosinophils may be responsible for the production of some of these mediators. Concomitantly with LTC_4, the urinary concentration of LTB_4 glucuronide, a urinary metabolite of LTB_4, increased in the AIA group after aspirin provocation. Thus, there may be a possibility that these mediators were partly generated from eosinophils by cell-to-cell interaction.[70] However, considering that there was no significant change in the serum level of eosinophil cationic protein,[78] urinary eosinophil-derived neurotoxin or 3-bromotyrosine,[71] which are biomarkers of eosinophil activation, during AIA acute reaction, this hypothesis of activated eosinophils involvement remains a matter of speculation. More importantly, basophils also may be partly responsible for the production of LTC_4 and histamine, but not PGD_2.[55,79]

Song *et al.* demonstrated that the urinary tetranor-PGDM concentrations were suppressed by inhibition with aspirin, but not by a selective inhibition of COX-2.[61] Interestingly, Daham *et al.* recently demonstrated that the urinary tetranor-PGDM concentration remains unchanged in both the AIA and ATA groups following the administration of the selective COX-2 inhibitor celecoxib.[68] Taken together, despite the fact that COX-1 is the dominant *in vivo* PGD_2 biosynthesis pathway, the precise mechanism underlying the PGD_2 overproduction through the pharmacological effect of COX-1 inhibitors in the AIA group remains unknown.

PG PRODUCTION VIA THE FREE RADICAL-MEDIATED "ISOPROSTANE PATHWAY"

Recently, it was reported that there is another pathway in which arachidonic acid is metabolized *in vivo* by a free radical-mediated mechanism to yield a series of PG-like compounds termed isoprostanes that is independent on the catalytic activity of the COX enzyme.[80] In contrast with COX-derived PGs, which is an optically pure form, the radical-mediated peroxidation of arachidonic acid generates a racemic mixture of PGs (Fig. 5). Thus, the presence of the enantiomer to COX-derived PG indicates that the PG is generated via a free radical-mediated mechanism.[81] The concentration of the PG enantiomer in urine is a reliable index of systemic isoprostane and lipid oxidation.[81,82] In particular, quantification of the urinary $PGF_{2}\alpha$ enantiomer (*ent*-$PGF_{2}\alpha$) constitutes a valuable tool for assessing oxidant stress *in vivo*.[82] As judged by the urinary *ent*-$PGF_{2}\alpha$ concentration, free radical-mediated PG generation is also involved in the pathophysiology of IgE-mediated anaphylaxis.[25] Therefore, we hypothesized that a free radical-mediated mechanism might be also responsible for the PGD_2 production which occurs during the AIA reaction. However, unexpectedly, there was no change in the urinary *ent*-$PGF_{2}\alpha$ concentrations after aspirin provocation in the AIA group.[25] It seems that PGD_2 overproduction *in vivo* in the AIA group after the aspirin provocation test is independent of the isoprostane pathway. More interestingly, recent studies on PG biosynthesis have demonstrated that nitric oxide nitrosylates cytosolic phospholipase A_2 ($cPLA_2$)[83] and COX-2,[84] resulting in the activation of these two enzymes and an increase in PG synthesis. These findings suggest that oxidative stress induces the post-translational modification of enzymes associated with eicosanoid biosynthesis.[85]

CONCLUSION AND CLINICAL RELEVANCE

This review article focuses on clinical significance of urinary biomarkers in AIA. We have analyzed a variety of biological samples, including serum, saliva,[24,86] sputum,[27] exhaled breath condensate[87,88] and bronchoalveolar lavage fluid[87,89] to assess the pathophysiology of allergic diseases such as AIA. To our knowledge, the biomarker of urinary LTE_4 is the only appropriately sensitive biomarker for the aspirin-induced asthma/sinus reaction. Christie *et al.*[90] have reported that AIA subjects exhibit selective hyperresponsiveness to LTE_4, but not to LTC_4, relative to that seen in ATA subjects. Interestingly, P2Y12, the adenosine diphosphate receptor, is responsible for the LTE_4-mediated activation and proliferation of mast cells, as well as amplification of allergic pulmonary inflammation.[91,92] Taken these findings together, it is suggested that a mechanism underlying AIA is that LTE_4-mediated signaling pathway may play an important role for the development of refractory asthma. In addition, new receptors for LTE_4 have recently been discovered. Their roles in CysLT signaling and related diseases, in particular AIA, need to be elucidated.[93,94] Single measurements of urinary LTE_4 concentrations do not allow a demonstration of the cellular source and target organ of CysLT production. We preliminarily compared the urinary LTE_4 concentrations in the same patients (n = 3) exhibiting the same extent of bronchoconstriction upon systemic challenge and inhalation challenge. The duration between the two different challenges was more than 2 weeks to avoid the refractory period in AIA. The extent of increase in the urinary LTE_4 concentration after systemic aspirin challenge was 3 to 10-fold higher than aspirin inhalation challenge (data not shown). This finding suggests that the site of CysLT production is not only the bronchi and lungs. Therefore, further quantification of the urinary PGD_2 metabolite concentrations are helping to elucidate how

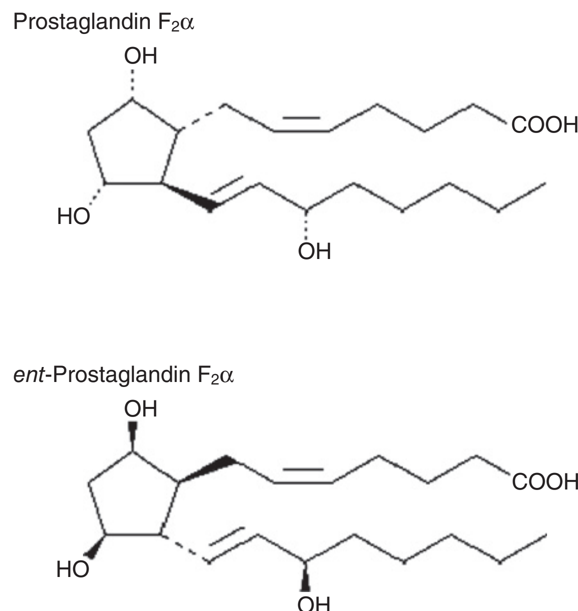

Fig. 5 Chemical structures of prostaglandin $F_{2}\alpha$ and *ent*-prostaglandin $F_{2}\alpha$.

mast cell activation is involved in the pathophysiology of AIA.[24,25,28,64,95] However, the precise mechanisms underlying the PGD_2 overproduction in the AIA group, which occurred despite the administration of a COX inhibitor, remains unknown. Paruchuri *et al.* have reported that LTE_4 activates human mast cells by a pathway that involves a cooperation between MK571-sensitive G protein coupled receptors (GPCRs) and peroxisome proliferator activated receptor γ (PPARγ), a nuclear receptor for dietary lipids. LTE_4 possesses a capacity for upregulating COX-2 expression and causing PGD_2 generation.[96] Furthermore, He *et al.*[85] have demonstrated that treatment of bone marrow-derived mast cells (BMMC) with PGD_2 reduces the ability of BMMC to generate LTC_4 upon calcium ionophore stimulation, but has little effect on LTB_4 generation. This effect can be reproduced by a selective agonist of the DP2 receptor, 15R-methyl-PGD_2 (15R-PGD_2). 15R-PGD_2 exerts its suppressive effect via a reduction in intracellular glutathione (GSH), a mechanism that involves the conjugation of its non-enzymatic breakdown product with GSH. Quantification of the new biomarker PGD_2 metabolite tetranor-PGDM provides a clinically useful tool for assessing mast cell activation *in vivo*. Urine samples afford a most non-invasive research tool for time-course measurements in a clinical study, and a lipidmics approach using biological fluid samples such as urine will provide further clinical data targeting mast cell activation, not only in allergic diseases, but also infectious diseases and cancer.

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